



Sporadic short temperature events cannot be neglected in predicting impacts of climate change on small insects



Liang Zhu^{a,b}, Lin Wang^a, Chun-Sen Ma^{a,*}

^a Climate Change Biology Research Group, State Key Laboratory for Biology of Plant Diseases and Insect Pests, Inst. of Plant Protection, Chinese Academy of Agricultural Sciences, No. 2 Yuanmingyuan West Road, Haidian District, CN-100193 Beijing, PR China

^b Institute of Plant and Environmental Protection, Beijing Academy of Agriculture and Forestry Sciences, No. 9 Shuguanghuayuan Middle Road, Haidian District, CN-100097 Beijing, PR China

ARTICLE INFO

Keywords:

Sitobion avenae
Extreme climate event
Heat recovery
Fluctuating temperature
Heat wave
Mild temperature

ABSTRACT

Climate warming is characterized by increase in extreme heat events (EHEs). EHEs and mild temperature periods alternate with each other and form complex climate scenarios. Among these scenarios, low-frequency and short-duration extreme heat events during long mild periods (sporadic short EHEs) and low-frequency and short-duration mild periods during long extreme heat events (sporadic short mild periods) commonly occur in nature. The biological effects of these two types of temperature events have not been thoroughly elucidated to date. To clarify the biological effects of these temperature events on organisms, we selected the English grain aphid, a globally important cereal pest, as our model system. We exposed aphids to simulated 24-h diurnal fluctuating temperatures, inserted these events during the wheat growing season and then investigated development, adult longevity, fecundity, survival, and demographic parameters. We found that sporadic short mild periods during a long EHE could improve their life history traits. Increasing the duration of mild periods from 1 day to 2 days did not significantly change their demographic performance. Sporadic short EHEs during a long mild period did not significantly affect vital rates, while increasing the duration of EHEs from 1 day to 2 days worsened the aphids' performance. We found that short mild episodes in the hot season may benefit small insects to buffer long duration heatwaves. We discussed how sporadic short mild periods during a long EHE supplied aphids a chance to recover from heat stress. Thus, we suggest that sporadic temperature events should be considered in population prediction of small insects under climate change and should be integrated into pest management.

1. Introduction

Extreme heat events (EHEs) are increasing in recent years under global warming (Rahmstorf and Coumou, 2011; Buckley and Huey, 2016) (Fig. 1), though they are at the tails of climate distribution (Bailey and van de Pol, 2016; IPCC, 2012; Smith, 2011). EHEs negatively impact individual's metabolism and other physiological processes (Denlinger and Yocum, 1998; Karl et al., 2011), thereby influencing populations (Garrahou et al., 2009; Ma et al., 2018; Rahmstorf and Coumou, 2011; Rubio-Portillo et al., 2016), communities (Gillespie et al., 2012; Ma et al., 2015b; Sentis et al., 2013; Sunday et al., 2012) and ecosystems (Welbergen et al., 2008; Wernberg et al., 2013). In nature, EHEs and periods of mild temperature with different durations and frequencies alternate and form complex fluctuating conditions and temporal arrangements. Among these various temperature fluctuating scenarios, low-frequency and short-duration extreme heat events

during long mild periods (which we defined as “sporadic short EHEs”), and low-frequency and short-duration mild periods during long extreme heat events (which we defined as “sporadic short mild periods”) account for large proportions (Fig. 2). However, the biological effects of these two types of temperature events have been largely neglected.

Long EHEs are frequently interrupted by sporadic short mild periods under field conditions. For example, 1-day mild periods accounted for 42.1% of all the mild temperature periods with different durations in the late occurrence of wheat aphids (May 15th – June 30th, 1970–2015) in 2 wheat production areas of China, and among all 1-day mild temperature periods, 38.2% occurred in low frequency ($< 1/6$) (Fig. 2A). Such mild periods of short duration may play an important role in relieving stress from long EHEs due to the organism's heat injury recovery mechanisms at mild temperatures (Bowler and Kashmeery, 1979; Malmendal et al., 2006; Rodríguez-Trelles et al., 2013). However, previous studies mainly focused on biological impacts of constant high

* Corresponding author.

E-mail address: machunsen@caas.cn (C.-S. Ma).

<https://doi.org/10.1016/j.jinsphys.2018.12.003>

Received 5 September 2018; Received in revised form 4 December 2018; Accepted 4 December 2018

Available online 05 December 2018

0022-1910/ © 2018 Elsevier Ltd. All rights reserved.

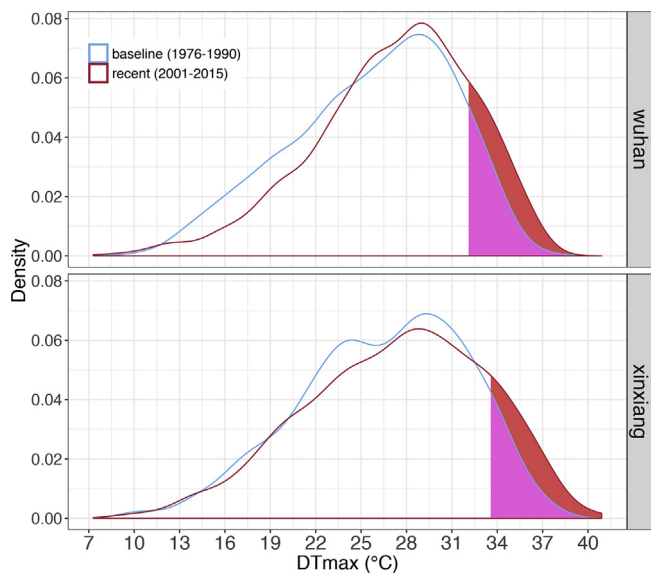


Fig. 1. Comparison of the distribution of daily maximum temperature (DTmax) between the baseline (1976–1990) period and the recent (2001–2015) period during April–June at Wuhan (30.47° N, 114.36° E) and Xinxiang (35.14° N, 113.77° E). The proportions of days with DTmax in the upper 10% of the baseline temperatures (Wuhan: 32.3 °C, Xinxiang: 33.7 °C) have increased recently (red shading). Daily maximum temperature records were downloaded from the China Meteorological Data Sharing Service System. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

temperatures (Angilletta et al., 2000; Asin and Pons, 2001; Davidson, 1944; Dean, 1974), lifetime consecutive diurnal fluctuating temperatures (Ma et al., 2015a, b; Paaijmans et al., 2013; Zhao et al., 2014) or EHEs with high frequencies or long durations (Bannerman and Roitberg, 2014; Ma et al., 2015a, b). Even in our recent study (Ma et al., 2018), although we noticed the importance of mild days in predicting the impacts of heat waves, we did not focus on sporadic short mild periods during a long EHE. Therefore, these published results do not reflect the biological role of sporadic short mild periods during a long EHE (Ma et al. 2018).

Sporadic short EHEs are very common under field conditions. EHEs lasting only one day accounted for 47.3% of all the EHEs with different durations in the early-middle stage of wheat aphid occurrence (April 1st – May 15th, 1970–2015) in 2 wheat growing areas of China, and 76.7% of all 1-day EHEs occurred in low frequency ($< 1/6$) (Fig. 2B). Such sporadic short EHEs may have little effect on organisms with long lifespans but they can change the biological performance of short lifespan species (Vincenzi et al., 2012), probably because such short EHEs account for a relatively larger proportion of their whole short lifespan. A single heat exposure had the ability to reduce fecundities of insects (Piyaphongkul et al., 2012; Zani et al., 2005; Zhang et al., 2015; Zhao et al., 2017). However, the effects of a single heat exposure at constant temperature might not reflect the real impacts of sporadic short EHEs due to ignoring the acclimation effects driven by gradually changing temperatures in diurnal fluctuation during extreme hot days (Ruel and Ayres, 1999; Kingsolver et al., 2009). The “Acclimation effect” refers to the phenomenon where a pretreatment at a substressful condition would increase tolerance for an extreme stress (see Angilletta, 2009). Although we used naturally fluctuating temperatures in a recent relevant study (Ma et al., 2018), we did not test the impacts of sporadic short EHEs. Thus, it remains unclear how this kind of climate scenario works under natural conditions.

To clarify the effect of sporadic short EHEs or mild periods on demographic performances of organisms, we selected *Sitobion avenae*, a globally distributed and destructive cereal pest (Dean, 1974; van

Emden and Harrington, 2007) as a model system. This aphid species has such features as a small body size, high reproduction rate, a short life cycle and is sensitive to temperature changes. Importantly, sporadic short EHEs commonly occurred in the early and middle stage of this aphids’ occurrence, and short mild periods often happened in the late stage of their occurrence, in 2 main wheat growing areas of China (Fig. 2A; B).

In this study, we investigated how sporadic short EHEs or mild periods affected the demography and fitness of *S. avenae*. We exposed English grain aphids to 0, 1, or 2 hot days or 0, 1, or 2 mild days in every 6-day cyclic period, respectively (Fig. 3), and then investigated life history traits and demographic parameters of the aphids until all tested aphids died. Our experiment allowed us to clarify the following questions. 1) Does short mild periods inserted into a period of consecutive hot days lead to a better biological performance of this aphid species and does increasing the duration of mild days (from 1 to 2 days) alter their performance significantly? 2) Do sporadic short EHEs occurring in consecutive mild days influence the life history traits of this species and how does increasing the duration of sporadic EHEs (from 1 to 2 days) affect these traits? We found that short mild periods during a long EHE could significantly improve demographic performances, but increasing the mild duration from 1 to 2 days did not affect the demographic performances significantly. Sporadic short EHEs did not reduce the life history performance significantly, but EHEs that were longer in duration did. We need consider these commonly but sporadically occurring short temperature events adequately in predicting the effect of climate change on natural and agricultural ecosystems.

2. Materials and methods

2.1. Stock rearing

Sitobion avenae were mixed populations collected from the long-term stock rearing in Institute of Plant Protection, Chinese Academy of Agricultural Sciences in Beijing, China. Aphids were reared on 5–20 cm high winter wheat seedlings (CA0045) in screen cages (60 × 60 × 60 cm³) at 22 ± 0.5 °C and 70 ± 10% RH with a 16: 8 (L: D) photoperiod in the rearing room. Our pilot experiment demonstrated that biological performances in the long-term laboratory population are similar to those in the field population reared in the lab for two generations. Winter wheats used in the study were planted in plastic pots in a separate room to prevent infestation from other insects.

2.2. Experimental design

We designed two separate experiments to test life history traits of English grain aphids under the scenarios of sporadic short mild period alternated with long EHEs or sporadic short EHEs alternated with long mild periods, respectively (Fig. 3). For the experiment to test the effect of the sporadic short mild period, we designed 3 temperature regimes, i.e., hot days: mild days = 6:0; 5:1; 4:2 to simulate 0, 1 or 2 mild days in every 6-day cyclic period. For the experiment to explore effect of sporadic short EHEs, we also designed 3 temperature regimes, i.e., hot days: mild days = 0:6; 1:5; 2:4 to simulate 0, 1 or 2 hot days in every 6-day cyclic period. To estimate possible variation due to different temperatures on the starting day, we conducted two parallel tests for each experiment by starting either with a hot day or with a mild day respectively (Fig. 3). For all tests, we examined the nymphal developmental time, adult longevity, lifetime fecundity, survival, and demographic parameters for each treatment, respectively.

We used 35 °C and 20 °C as the daily maximum and minimum temperatures for hot days, 28 °C and 13 °C as the daily maximum and minimum temperatures for mild days, respectively. We applied a sine curve to simulate the diurnal fluctuations with a magnitude of 15 °C. All the simulated temperature parameters are based on field temperature records and temperature responses of aphids in our previous studies

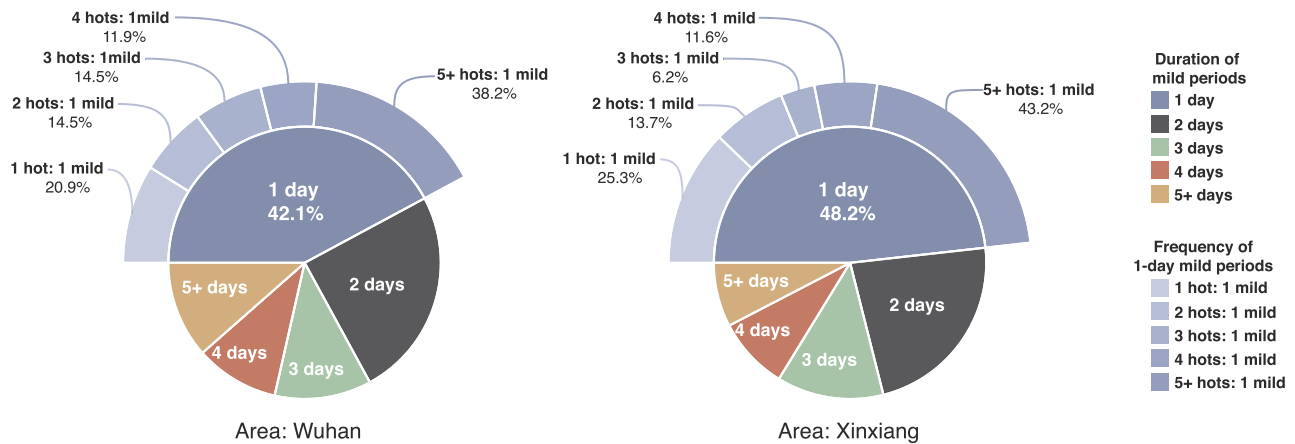
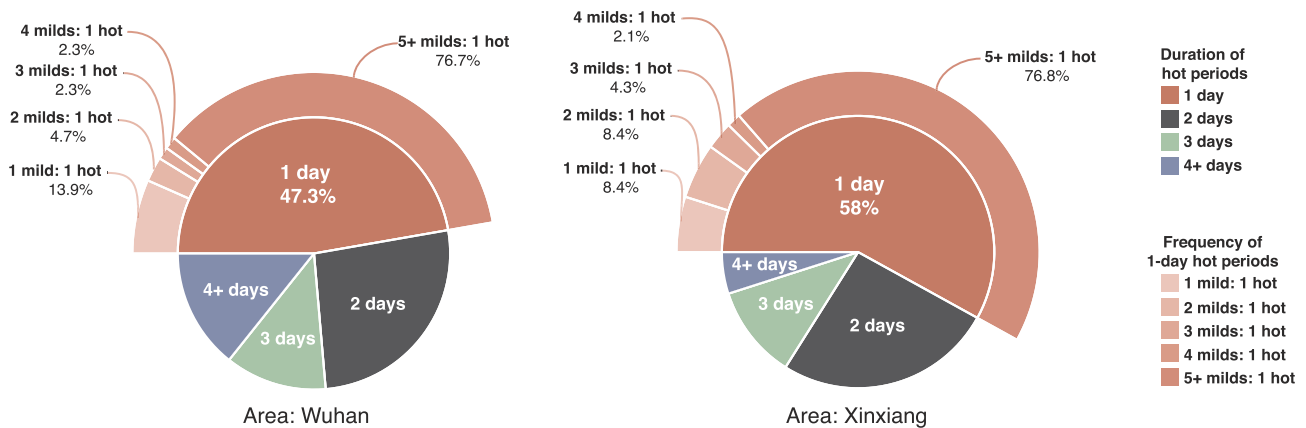
A: Distribution of consecutive mild periods with different durations and frequencies in hot season for cereal aphids**B: Distribution of consecutive hot periods with different durations and frequencies in mild season for cereal aphids**

Fig. 2. Proportional distribution of consecutive mild periods with different durations and frequencies in hot seasons (A) or consecutive hot periods with different durations and frequencies in mild seasons (B) for cereal aphids (April 1st to June 30th, 1971–2015) in 2 wheat-growing areas of China. In panel A the pie charts show the proportion of consecutive mild periods with different durations, and the donut charts show the different proportions of consecutive hot periods alternating with 1-day mild periods during the hot season for cereal aphids (May 16th to June 30th). In panel B the pie charts show the proportion of consecutive hot periods with different durations, and the donut charts show the different proportions of consecutive mild periods alternating with 1-day hot periods during the mild season for cereal aphids (April 1st to May 15th). We used the duration of consecutive hot/mild periods before the 1-day mild/hot period to indicate the frequency of the 1-day mild/hot period in the season (donut charts). Hot days occurred when the daily maximum temperature was $\geq 30^\circ\text{C}$, while mild days occurred when the daily maximum temperature was $\leq 28^\circ\text{C}$. It was shown that mild/hot periods that were short in duration (1 day) accounted for the largest proportion in the hot/mild season, and most of them were in low frequency (5+ hot days: 1 mild day or 5+ mild days: 1 hot day).

(Ma et al., 2018; Zhao et al., 2017; Zhao et al., 2014).

2.3. Experimental protocol

We conducted demographic experiments to discover the impacts of sporadic short temperature events on the life history traits and demographic parameters of aphids. In both experiments of sporadic short mild periods and EHEs, sufficient adult aphids were collected from stock rearing and allowed to produce new nymphs within 6 h. Meanwhile, we prepared plastic rearing tubes (diameter 1.5 cm, length 5.5 cm) for rearing the tested aphids. We filled each tube to one third with 0.6% agar solution and then inserted a newly excised wheat leaf to the cool agar medium to keep the leaf fresh. We transferred one new nymph to the leaf of each tube for feeding and then covered the tube with a piece of gauze. For each temperature treatment, we tested over 36 new nymphs (0–6 h old). We exposed aphids together with their rearing tubes to the temperature regimes according to our experimental design. We renewed the wheat leaves every day and the rearing tubes together with the agar medium every third day to keep the leaves fresh.

For the nymphs, we checked each aphid twice a day at 8:00 am and 8:00 pm, to record its survival status and instar stage identified by its exuvia. For the adult period, we observed each adult once a day at 8:00 am, to record its survival status, numbers of newly born nymphs and then removed dead adults and new offspring. We calculated the nymphal developmental time as the duration from birth to the last day of the fourth instar, adult longevity as the total number of days from adult emergence to death, and lifetime fecundity as the total offspring numbers produced during the whole adult stage.

We moved aphids and rearing tubes between the hot day chamber and the mild day chamber to expose tested aphids to designed temperature regimes. The temperature regimes were controlled with climatic chambers (PQX, Laifu Ltd., Ningbo, China; accuracy: $\pm 0.5^\circ\text{C}$), in which the relative humidity was 50–80% and the photoperiod was 16 h light: 8 h dark.

2.4. Statistical analysis

We identified error distributions of life history traits, i.e.,

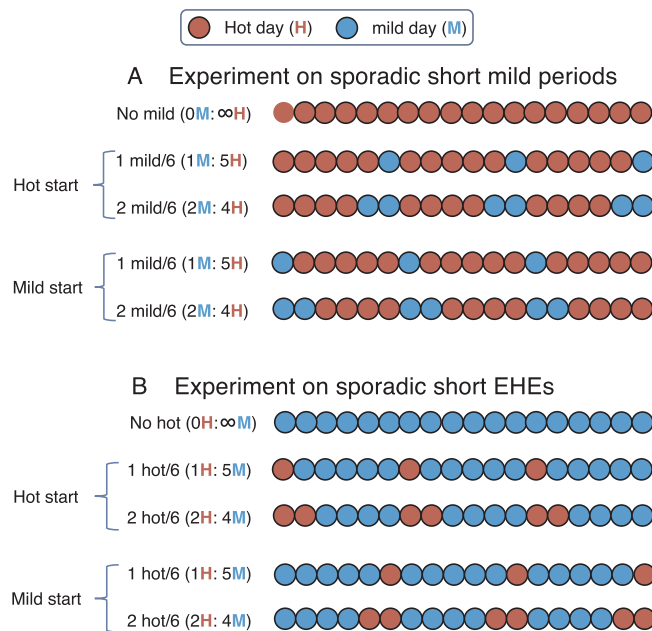


Fig. 3. Experimental designs to test the impacts of sporadic short mild periods (A), and extreme heat events (EHEs) (B) on life history traits and demographic parameters. Red and blue circles represent hot days and mild days respectively. A hot day or mild day was defined as the daily fluctuating temperature range from 20 °C to 35 °C or from 13 °C to 28 °C, respectively. ‘Hot start’ or ‘mild start’ indicates the first experimental day was a hot day or a mild day, respectively. ‘No mild’, ‘1 mild/6’, or ‘2 mild/6’ in Fig. 3A means 0, 1, or 2 mild days in every 6-day cyclic period respectively. ‘No hot’, ‘1 hot/6’, or ‘2 hot/6’ in Fig. 3B means 0, 1, or 2 hot days in every 6-day cyclic period respectively. Aphids were exposed to different treatments until they died and only 18 days are shown here to save space.

development time, adult longevity, and lifetime fecundity, by using ‘fitdistr’ and ‘AIC’ functions from the ‘car’ and ‘MASS’ packages in R. We used generalized linear models (GLM) with gamma distributed errors to analyze the effect of 2 fixed factors (mild or hot days and starting patterns) and their interactions on life history traits, with the ‘glm’ function and ‘car’ package in R. We further analyze how different mild or hot days, starting patterns, and their interactions affected the survival of aphids, by using the Cox proportional hazard model in the ‘survival’ and ‘car’ packages in R. The multiple comparisons based on the GLM or Cox model between different treatments were conducted by using ‘Tukey’ method, in the ‘glht’ function from the ‘multcomp’ package in R.

Demographic parameters, i.e., the net reproductive rate (R_0), generation time (G) and the intrinsic rate of increase (r_m) for each treatment were estimated by the following equations: $R_0 = \sum l_x \cdot m_x$, $G = \sum l_x \cdot m_x \cdot X / \sum l_x \cdot m_x$, $r_m = \ln(R_0) / G$, where ‘ X ’ is the age of aphids, ‘ l_x ’ is the proportional survival of aphids at age ‘ x ’, and ‘ m_x ’ is the number of offspring per female at age ‘ x ’. We calculated means and corresponding 95% confidence intervals for the demographic parameters of aphids by using the bootstrap procedure and compared the different treatments in ‘Kruskal-Wallis’ method using the ‘sample’ function and the ‘kruskal’ function from the ‘agricolae’ package in R.

3. Results

3.1. Development

For the experiment on sporadic short mild periods, neither a different number of mild days nor starting patterns affected the developmental time, but their interaction did (Table 1). When the test started with a hot day, the one mild day treatment led to slower nymphal

development than the control (no mild days) (Fig. 4A). While in the test that started with a mild day, nymphal developmental times among all treatments were not different (Fig. 4A).

For the experiment on sporadic short EHEs, a different number of hot days, starting patterns and their interactions all affected the developmental time of nymphs (Table 2). No matter if it started with a hot or mild day, one hot day resulted in faster nymphal development than the control (no hot days) (Fig. 4B). In addition, two hot days accelerated the nymphal development if the test started with a mild day, but not so if the test started with a hot day (Fig. 4B).

3.2. Longevity

For the experiment on sporadic short mild periods, a different number of mild days affected adult longevity (Table 1), but starting patterns did not (Table 1). No interaction existed between these two factors (Table 1). No matter if it started with a hot or mild day, adult longevity was higher in the 1 or 2 mild day treatment than the control (no mild days), but the traits between 1 and 2 mild day treatments were not different (Fig. 4A).

For the experiment on sporadic short EHEs, a different number of hot days affected adult longevity (Table 2), but starting patterns did not (Table 2). No interaction existed between these two factors (Table 2). Regardless if it started with a hot or mild day, adult longevity was higher in the 1 or no hot day treatment than that in the 2 hot day treatment, but the traits in the 1 and no hot day treatments were similar (Fig. 4B).

3.3. Fecundity

For the experiment on sporadic short mild periods, a different number of mild days affected the lifetime fecundity of adults (Table 1), but starting patterns did not (Table 1). No interaction existed between these two factors (Table 1). Regardless if the experiment started with a hot or mild day, adult fecundity was higher in the 1 or 2 mild day treatment than the control (no mild days), but the traits between the 1 and 2 mild day treatments were similar (Fig. 4A).

For the experiment on sporadic short EHEs, a different number of hot days affected the lifetime fecundity of adults (Table 2), but starting patterns did not (Table 2). A weak interaction existed between these two factors (Table 2). When the test started with a hot day, adult fecundity was higher in the 1 or no hot day treatment than that in the 2 hot day treatment, but the traits in the 1 and no hot day treatments were not different (Fig. 4B). While the test started with a mild day, no significant difference existed in the traits between each pair of treatments (Fig. 4B).

3.4. Survival

For the experiment on sporadic short mild periods, a different number of mild days affected the survival of aphids (Table 1), but starting patterns did not (Table 1). No interaction existed between these two factors (Table 1). No matter if it started with a hot or mild day, the survival of aphids was higher in the 1 or 2 mild day treatment than the control (0 mild days) (Table 3; Fig. 5A), but were not different between the 1 and 2 mild day treatments (Table 3; Fig. 5A).

For the experiment on sporadic short EHEs, a different number of hot days affected the survival of aphids (Table 2), but starting patterns did not (Table 2). A weak interaction existed between these two factors (Table 2). When the test started with a hot day, the survival of aphids was not lower in the 1 hot day treatment than the control (0 hot days) (Table 4; Fig. 5B), while it was lower in the 2 hot day treatment than that in the 1 or no hot day treatment (Table 4; Fig. 5B). When the test started with mild day, the survival of aphids were similar between the 1 and no hot day treatments, or between the 1 and 2 hot day treatments (Table 4; Fig. 5B), but this trait was lower in the 2 hot day treatment

Table 1

Effects of sporadic mild periods and their starting patterns on life history traits of the English grain aphid. Mild days include 0, 1, or 2 days. The starting pattern included starting from either a hot day or a mild day. Statistic results are χ^2 of GLMs or Cox proportional hazard models and corresponding p-values. P-values with significant values are indicated in bold. One or two asterisks after the p-value represent the factor in the model affects traits significantly ($P < 0.05$ or $P < 0.01$).

	df	Development time	Longevity	Fecund	Survival
Mild days (MD)	2	$\chi^2 = 2.41$, $P = 0.300$	$\chi^2 = 75.33$, $P < 0.0001^{**}$	$\chi^2 = 44.49$, $P < 0.0001^{**}$	$\chi^2 = 69.86$, $P < 0.0001^{**}$
Starting pattern (SP)	1	$\chi^2 = 0.70$, $P = 0.403$	$\chi^2 = 0.06$, $P = 0.803$	$\chi^2 = 0.03$, $P = 0.864$	$\chi^2 = 0.004$, $P = 0.947$
MD*SP	2	$\chi^2 = 6.77$, $P = 0.034^*$	$\chi^2 = 0.03$, $P = 0.983$	$\chi^2 = 0.241$, $P = 0.886$	$\chi^2 = 0.381$, $P = 0.826$
Error	280				

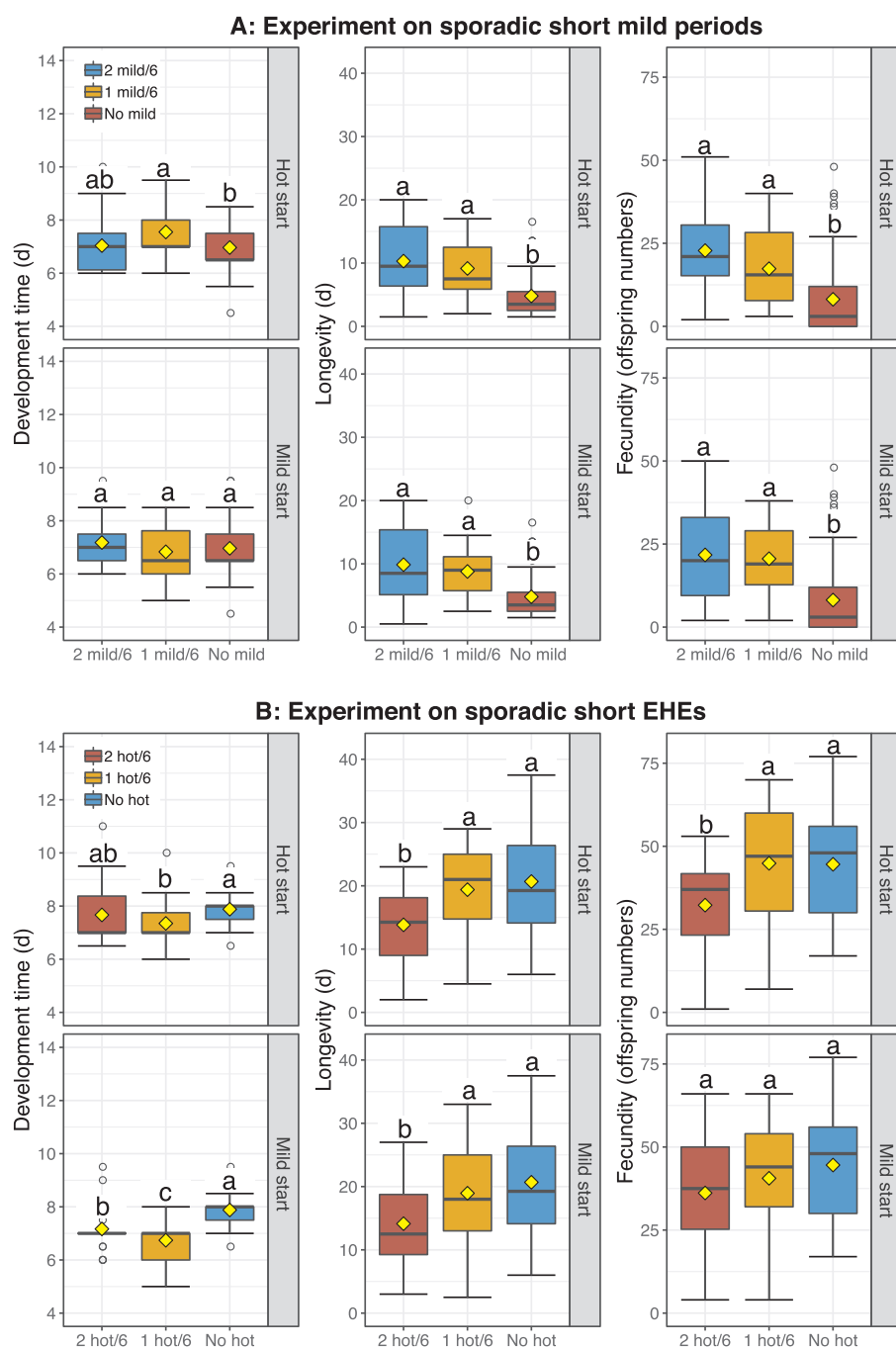


Fig. 4. Comparisons of life history traits of the English grain aphid during different sporadic short mild periods (A) or extreme heat events (EHEs) (B). Different letters represent significant differences between treatments. Yellow diamonds in the middle of box plot indicate the mean value. A legend is located in the first panel of each experiment to show the treatments. The meaning of treatment names refers to Fig. 3. The text in the gray strips on the right shows the starting patterns.

Table 2

Effects of sporadic extreme heat events (EHEs) and their starting patterns on life history traits and the survival of the English grain aphids. Hot days included 0, 1, or 2 days. The starting pattern included starting from either a hot day or a mild day. Statistic results are χ^2 of GLMs or Cox proportional hazard models and corresponding p-values. P-values with significant values are indicated in bold. One or two asterisks after the p-value represent the factor in the model affects traits significantly ($P < 0.05$ or $P < 0.01$).

	df	Development time	Longevity	Fecund	Survival
Hot days (HD)	2	$\chi^2 = 40.75$, $P < 0.0001^{**}$	$\chi^2 = 24.16$, $P < 0.0001^{**}$	$\chi^2 = 10.60$, $P = 0.005^{**}$	$\chi^2 = 38.53$, $P < 0.0001^{**}$
Starting pattern (SP)	1	$\chi^2 = 6.61$, $P = 0.010^*$	$\chi^2 = 0.002$, $P = 0.966$	$\chi^2 = 0.01$, $P = 0.924$	$\chi^2 = 1.66$, $P = 0.197$
HD*SP	2	$\chi^2 = 7.16$, $P = 0.028^*$	$\chi^2 = 0.063$, $P = 0.969$	$\chi^2 = 1.53$, $P = 0.466$	$\chi^2 = 1.64$, $P = 0.439$
Error	178				

than the control (0 hot days) (Table 4; Fig. 5B).

3.5. Demographic parameters

For the experiment on sporadic short mild periods, no matter if it started with a hot or mild day, the net reproduction rate (R_0), generation times (G) or intrinsic rate of increase (r_m), was higher in the 1 or 2 mild day treatment than the control (0 mild days) (Fig. 6A). Starting patterns affected comparison of each parameter between the 1 and 2 mild day treatments (Fig. 6A).

For the experiment on sporadic short EHEs, no matter if it started with a hot or mild day, R_0 or G was higher in the control (0 hot days) than in the 1 or 2 hot day treatment (Fig. 6B). When the test started with a hot day, r_m were not different between the 0 and 1 hot day treatments; it was higher in the 0 or 1 hot day treatment than in the 2 hot day treatment (Fig. 6B).

4. Discussion

Sporadic short temperature events often occur under climate change, such as sporadic short EHEs in spring or sporadic short mild periods in summer. However, these events are neglected due to their low frequency and short duration but may be important for short-lived species. In this study, we tested the impacts of sporadic short temperature events on a small global pest, the English grain aphid. We found that life history traits, such as fecundity and adult longevity, and its population fitness, such as survival, R_0 , and r_m , were improved by sporadic short mild periods during a long EHE compared with consecutive hot days. In addition, increasing mild duration did not significantly change the life history traits of this insect. On the other hand, sporadic short EHEs during a long mild period did not significantly affect vital rates, such as fecundity, adult longevity and survival. Increase duration of EHEs resulted in worse performances of this insect species.

4.1. Sporadic short mild periods significantly buffered consecutive heat stress

Insects could relieve stress from a heat wave if they subsequently received frequent or long mild periods of several hours every day (Bowler and Kashmeery, 1979; Davis et al., 2006; Zhao et al., 2014) or a

few days within several days (Ma et al., 2018; Sentis et al., 2013). However, our new results suggested that even sporadic short mild periods helped insects to relieve heat stress and improve their fitness significantly (Fig. 4A; Fig. 5A; Fig. 6A; Table 3). Sporadic short mild periods did not reduce the mean temperatures the aphids experienced in the hot season significantly (Table S1), and this reduction by less than 1 °C could not change the development and fecundity of *S. avenae* significantly as inferred from Dean's study (1974b). We also compared the simulated performances driven by the aphids' thermal performance curve (Ma, unpublished data) to the tested results and found that they are far away. Thus, short-step thermal driving performances could not be easily combined into an integrated performance by a whole temperature event, though they might contribute to our tested results to some extent. Therefore, the effects of sporadic short mild days in buffering heat stress might be attributed to other more relative factors, such as physiological and biochemical responses (Colinet et al., 2015), besides the thermal driving factor. A short mild period in the middle of consecutive hot days might interrupt the continuous injury and prevent irrecoverable damage (Rensing and Ruoff, 2009; Jaenicke, 1991; Walter et al., 2010), allowing the opportunity to reestablish metabolite homeostasis (Hoffmann, 1984; Neven 2000; Hochachka and Somero, 1984; MacMillan et al., 2012). In addition, some essential nutrients might be derived during the short mild period and carried over to relieve the next stress experienced by the insects (Hansen and Moran, 2011; Oliver et al., 2010).

Moreover, aphids in our study did not perform better when the duration of the mild period in every cyclic time increased from 1 day to 2 days (Fig. 4A; Fig. 5A; Table 3), which indicated that the presence of a sporadic short mild period, rather than its duration, was important for the insects to relieve heat stress. An extra duration of the mild period might relax organisms' vigilance against the later extreme heat events. For instance, accumulated anti-stress substances induced by the last heat stress (Kristensen et al., 2003) might be dissolved thoroughly during the extra mild period. In addition, we speculate that the short-term repairing pattern might be an advantage to small organisms with short lifespans in adaptive evolution, as they need to be able to fully recover themselves in a short period of time. Small organisms with short-term repairing patterns might survive better than those with restricted long-term repairing mechanisms to respond to rapid climate change. Therefore, some insects' success in surviving under extreme temperature conditions in nature might be attributed to such rapid

Table 3

Multiple comparisons using the Tukey method based on the Cox model for survival of the English grain aphid in different treatments of sporadic short mild periods. P-values with significant values are indicated in bold. One or two asterisks after the p-value represent traits between different treatments are significant different ($P < 0.05$ or $P < 0.01$).

Start pattern	Treatment pairs	Estimate	Standard error	Z	P	df
Hot start	1-0 rec	-0.6572	0.1955	-3.361	0.00228^{**}	2, 140
	2-0 rec	-1.161	0.2162	-5.369	< 0.001^{**}	
	2-1 rec	-0.5038	0.2569	-1.961	0.1193	
Mild start	1-0 rec	-0.7508	0.1953	-3.844	0.000307^{**}	2, 140
	2-0 rec	-0.9388	0.2096	-4.479	< 1.00E-04^{**}	
	2-1 rec	-0.1881	0.2496	-0.753	0.728424	

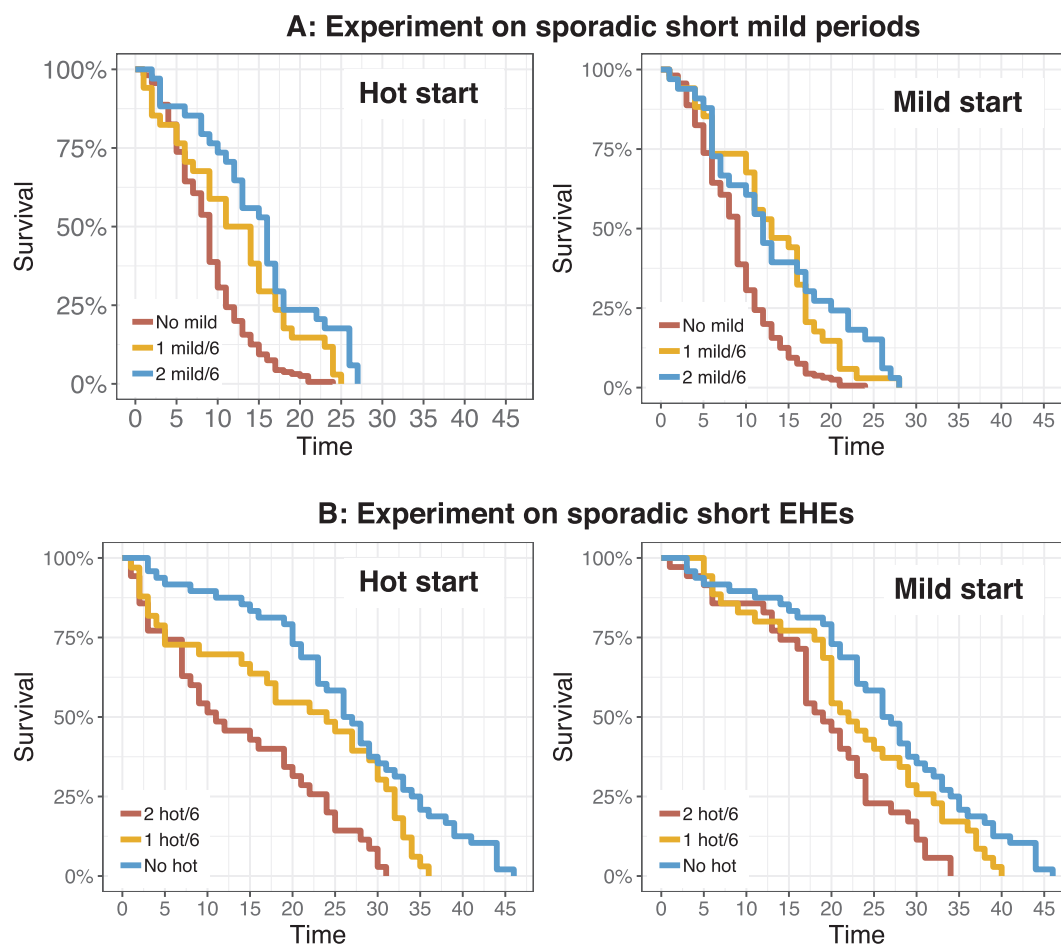


Fig. 5. Survival curves of the English grain aphid under different treatments of sporadic short mild periods (A) or extreme heat events (EHEs) (B). Legends in each panel show the different treatments. The meaning of treatment names refers to Fig. 3. The text in the top right corner of each panel shows the starting patterns.

Table 4

Multiple comparisons using the Tukey method based on the Cox model for survival of the English grain aphid in different treatments of sporadic short extreme heat events (EHEs). P-values with significant values are indicated in bold. One or two asterisks after the p-value represent traits between different treatments are significant different ($P < 0.05$ or $P < 0.01$).

Start pattern	Treatment pairs	Estimate	Standard error	Z	P	df
Hot start	1-0 hot	0.5487	0.2408	2.279	0.0588	2, 80
	2-0 hot	1.283	0.2526	5.079	< 0.001**	
	2-1 hot	0.7343	0.2537	2.895	0.0104*	
Mild start	1-0 hot	0.4131	0.2306	1.791	0.172	2, 98
	2-0 hot	0.9022	0.2405	3.751	< 0.001**	
	2-1 hot	0.4891	0.2449	1.997	0.113	

repairing patterns to make use of the irregular and short mild periods.

4.2. Negative impacts of sporadic short EHEs could be repaired

Short high temperature exposure, even with low frequency, might affect insects' biological performance significantly (Liang et al., 2014; Sentis et al., 2013; Zhang et al., 2013). However, our results suggest different conclusions. The short EHEs that lasted for 1 day did not have a significant impact on life history traits such as fecundity, longevity and survival of the aphids (Fig. 4B; Fig. 5B; Table 4). In previous heat-shock studies, insects were directly moved from constant rearing temperatures (e.g., 23–25 °C) to extreme high temperatures (e.g., 38–40 °C) (Liang et al., 2014; Zhang et al., 2013). However, in our present study,

aphids were treated in diurnal fluctuating temperature regimes in which temperatures increased gradually, and consequently, the impacts of stress on the aphids might be smaller than moving them directly from 20 °C to 35 °C (Zhao et al., 2014) due to thermal acclimation. Moreover, the temperature settings in the hours during mild periods after stress are different between previous studies and ours, where the former used constant mild temperatures (Liang et al., 2014; Sentis et al., 2013; Zhang et al., 2013), while the later used diurnal fluctuating temperatures with a mild mean (20.5 °C). Insects could perform better in fluctuating temperatures within an optimal temperature zone than in constant temperatures (Bross et al., 1999; Colinet et al., 2015). The potentially enhanced recovery from the fluctuating conditions might offset greater impacts of a short EHE on organisms in our present study, leading to insignificant biological results caused by sporadic short EHEs.

Furthermore, increasing the duration of EHEs from 1 to 2 days significantly reduced the performance of the aphids (Fig. 4B; Fig. 5B; Table 4), as is consistent with results of Ma et al. (2018) or Sentis et al. (2013), in which aphids also performed worse when the proportion of hot days increased. It could be inferred from these results that EHEs might not generate significant effects unless their frequencies exceed a certain threshold. Thus, EHEs might trigger a negative threshold-like response in the organisms in our study (Bailey and van de Pol, 2016). When the duration or frequency of EHEs passed the inflection point of the organisms' response curve, the accumulated injury on them was irreparable, leading to a significant decrease in their fitness. From another standpoint, EHEs of a sufficient duration might affect the aphids' performances by decreasing the density of their obligate symbionts,

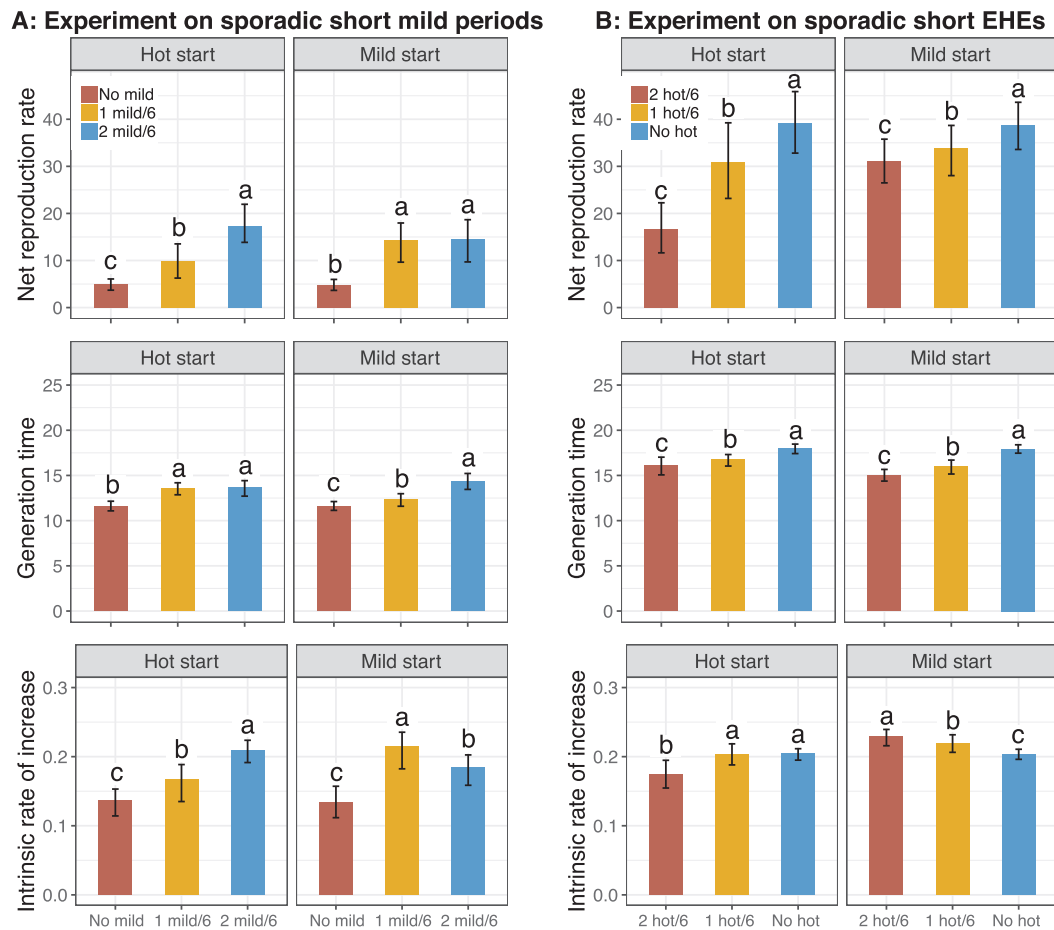


Fig. 6. Comparison of demographic parameters of the English grain aphid during different treatments of sporadic short mild periods (A) or extreme heat events (EHEs) (B). Different letters above each column represent significant differences between treatments. Meaning of treatment names refers to Fig. 3. Legends in the first panel of each subgraph show the different treatments. The text in gray strips on the top of each panel shows the starting pattern. Error bars indicate 95% confidence intervals.

which could supply essential amino acids for their hosts but would not be able to do that in a hot environment for long (Burke et al., 2010; Russell and Moran, 2006).

4.3. Significance and potential applications

Climate change is complicated and its impact on organisms is difficult to predict (Cerdá and Retana, 2000; Easterling et al., 2000). To assess the biological effects of realistic natural temperature under climate change, scientists have never stopped improving relative studies by extending temperature conditions from constant to fluctuating temperature, and further to EHEs (Colinet et al., 2015; Luber and McGeehin, 2008; Walther et al., 2002). EHEs with high frequency or long duration have attracted the attention of ecologists and negative effects have been found in many systems (Bannerman and Roitberg, 2014; Bauerfeind and Fischer, 2014; Ma et al., 2018; Ma et al., 2015a,b). However, sporadic short EHEs and mild periods are often neglected, probably because they are thought to have minor effects. In fact, they may occur more often than the frequent or long ones in some seasons, such as short EHEs in spring and short mild periods in hot summer. Our study suggested that these sporadic short events, especially short mild periods, could produce important ecological effects and deserve more attention in predicting the impacts of climate change. Our findings indicate that only one mild day in summer did not change the seasonal mean temperature much, but effectively relieved organisms from heat stress. Thus, in order to predict the effect of EHEs accurately in the context of climate change, we should consider not only

the temperature amplitude, duration and frequency but also the detailed fluctuating sequences of temperatures and biological responses of different species to them. In conclusion, the short mild episode in hot season or short hot episode in mild season may produce an impact on small insects, and they should be considered an essential part for pest prediction and for future integrated management.

Acknowledgements

We thank Dr. Gang Ma for his constructive discussion during the whole research procedure and the providing experimental materials.

Funding

This research was mainly financially supported by research grants of the National Natural Science Foundation of China (31620103914, 31772156, 31400350) and Innovation Program of Chinese Academy of Agricultural Sciences.

Compliance with ethical standards

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The authors declare that they have no conflict of interest.

Author contribution statement

CSM conceived and designed research. LZ and LW conducted experiments. LZ analyzed the data. CSM and LZ wrote the manuscript. All authors read and approved the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jinsphys.2018.12.003>.

References

- Angilletta, M.J., 2009. Thermal Adaptation: A Theoretical and Empirical Synthesis. Oxford University Press, New York, pp. 126–156.
- Angilletta, M.J., Winters, R.S., Dunham, A.E., 2000. Thermal effects on the energetics of lizard embryos: implications for hatchling phenotypes. *Ecology* 81, 2957–2968.
- Asin, L., Pons, X., 2001. Effect of high temperature on the growth and reproduction of corn aphids (Homoptera: Aphididae) and implications for their population dynamics on the Northeastern Iberian Peninsula. *Environ. Entomol.* 30, 1127–1134.
- Bailey, L.D., van de Pol, M., 2016. Tackling extremes: challenges for ecological and evolutionary research on extreme climatic events. *J. Anim. Ecol.* 85, 85–96.
- Bannerman, J.A., Roitberg, B.D., 2014. Impact of extreme and fluctuating temperatures on aphid–parasitoid dynamics. *Oikos* 123, 89–98.
- Bowler, K., Kashmeery, A., 1979. Recovery from heat injury in the blowfly, *Calliphora erythrocephala*. *J. Therm. Biol.* 4, 197–202.
- Bross, P., Corydon, T.J., Andresen, B.S., Jørgensen, M.M., Bolund, L., Gregersen, N., 1999. Protein misfolding and degradation in genetic diseases. *Hum. Mutat.* 14, 186–198.
- Buckley, L.B., Huey, R.B., 2016. Temperature extremes: geographic patterns, recent changes, and implications for organismal vulnerabilities. *Global Change Biol.* 22, 3829–3842.
- Burke, G.R., McLaughlin, H.J., Simon, J.C., Moran, N.A., 2010. Dynamics of a recurrent *Buchnera* mutation that affects thermal tolerance of pea aphid hosts. *Genetics* 186, 367–372.
- Cerdá, X., Retana, J., 2000. Alternative strategies by thermophilic ants to cope with extreme heat: individual versus colony level traits. *Oikos* 89, 155–163.
- Colinet, H., Sinclair, B.J., Vernon, P., Renault, D., 2015. Insects in fluctuating thermal environments. *Annu. Rev. Entomol.* 60, 123–140.
- Davidson, J., 1944. On the relationship between temperature and rate of development of insects at constant temperatures. *J. Anim. Ecol.* 13, 26–38.
- Davis, J., Radcliffe, E., Ragsdale, D., 2006. Effects of high and fluctuating temperatures on *Myzus persicae* (Hemiptera: Aphididae). *Environ. Entomol.* 35, 1461–1468.
- Dean, G.J., 1974. The overwintering and abundance of cereal aphids. *Ann. Appl. Biol.* 76, 1–7.
- Denlinger, D.L., Yocum, G.D., 1998. Physiology of heat sensitivity. In: Hallman, G.J., Denlinger, D.L. (Eds.), *Temperature Sensitivity in Insects and Application in Integrated Pest Management*. Westview Press, Oxford, pp. 7–57.
- Easterling, D.R., Meehl, G.A., Parmesan, C., Changnon, S.A., Karl, T.R., Mearns, L.O., 2000. Climate extremes: observations, modeling, and impacts. *Science* 289, 2068–2074.
- Garrabou, J., Coma, R., Bensoussan, N., Bally, M., Chevaldonne, P., 2009. Mass mortality in Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave. *Global Change Biol.* 15, 1090–1103.
- Gillespie, D.R., Nasreen, A., Moffat, C.E., Clarke, P., Roitberg, B.D., 2012. Effects of simulated heat waves on an experimental community of pepper plants, green peach aphids and two parasitoid species. *Oikos* 121, 149–159.
- Hansen, A.K., Moran, N.A., 2011. Aphid genome expression reveals host–symbiont co-operation in the production of amino acids. *Proc. Natl. Acad. Sci. U.S.A.* 108, 2849–2854.
- Hochachka, P., Somero, G., 1984. Temperature adaptation. *Biochemical Adaptation* 355–449.
- Hoffmann, K.H., 1984. Metabolic and enzyme adaptation to temperature. In: *Environmental Physiology and Biochemistry of Insects*. Springer, pp. 1–32.
- IPCC, 2012. Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation. A Special Report of Working Groups I and II of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK, and New York, NY, USA.
- Jaenicke, R., 1991. Protein Stability and Molecular Adaptation to Extreme Conditions. Springer, Berlin Heidelberg, pp. 291–304.
- Karl, I., Stoks, R., De Block, M., Janowitz, S.A., Fischer, K., 2011. Temperature extremes and butterfly fitness: conflicting evidence from life history and immune function. *Global Change Biol.* 17, 676–687.
- Kingsolver, J.G., Ragland, G.J., Diamond, S.E., 2009. Evolution in a constant environment: thermal fluctuations and thermal sensitivity of laboratory and field populations of *Manduca sexta*. *Evolution* 63, 537–541.
- Kristensen, T.N., Sørensen, J.G., Loeschcke, V., 2003. Mild heat stress at a young age in *Drosophila melanogaster* leads to increased Hsp70 synthesis after stress exposure later in life. *J. Genet.* 82, 89–94.
- Liang, L.N., Zhang, W., Ma, G., Hoffmann, A.A., Ma, C.S., 2014. A single hot event stimulates adult performance but reduces egg survival in the oriental fruit moth, *Grapholitha molesta*. *PLoS One* 9, e116339.
- Luber, G., McGehehin, M., 2008. Climate change and extreme heat events. *Am. J. Prev. Med.* 35, 429–435.
- Ma, C.S., Wang, L., Zhang, W., Rudolf, V.H., 2018. Resolving biological impacts of multiple heat waves: interaction of hot and recovery days. *Oikos* 127, 622–633.
- Ma, G., Hoffmann, A.A., Ma, C.S., 2015a. Daily temperature extremes play an important role in predicting thermal effects. *J. Exp. Biol.* 218, 2289–2296.
- Ma, G., Rudolf, V.H.W., Ma, C.S., 2015b. Extreme temperature events alter demographic rates, relative fitness, and community structure. *Global Change Biol.* 21, 1794–1808.
- MacMillan, H.A., Williams, C.M., Staples, J.F., Sinclair, B.J., 2012. Reestablishment of ion homeostasis during chill-coma recovery in the cricket *Gryllus pennsylvanicus*. *Proc. Natl. Acad. Sci. U.S.A.* 109, 20750–20755.
- Malmendal, A., Overgaard, J., Bundy, J.G., Sørensen, J.G., Nielsen, N.C., Loeschcke, V., Holmstrup, M., 2006. Metabolomic profiling of heat stress: hardening and recovery of homeostasis in *Drosophila*. *Am. J. Physiol.-Reg. I.* 291, 205–212.
- Neven, L.G., 2000. Physiological responses of insects to heat. *Postharvest Biol. Technol.* 21, 103–111.
- Oliver, K.M., Degnan, P.H., Burke, G.R., Moran, N.A., 2010. Facultative symbionts in aphids and the horizontal transfer of ecologically important traits. *Entomology* 55, 247–266.
- Paaijmans, K.P., Heinig, R.L., Seliga, R.A., Blanford, J.I., Blanford, S., Murdock, C.C., Thomas, M.B., 2013. Temperature variation makes ectotherms more sensitive to climate change. *Global Change Biol.* 19, 2373–2380.
- Piyaphongkul, J., Pritchard, J., Bale, J., 2012. Can tropical insects stand the heat? a case study with the brown planthopper *Nilaparvata lugens*. *PLoS One* 7, e29409.
- Rahmstorf, S., Coumou, D., 2011. Increase of extreme events in a warming world. *Proc. Natl. Acad. Sci. U.S.A.* 108, 17905–17909.
- Rensing, L., Ruoff, P., 2009. Temperature effect on entrainment, phase shifting, and amplitude of circadian clocks and its molecular bases. *Chronobiol. Int.* 19, 807.
- Rodríguez-Trelles, F., Tarrío, R., Santos, M., 2013. Genome-wide evolutionary response to a heat wave in *Drosophila*. *Biol. Lett.* 9, 20130228.
- Rubio-Portillo, E., Izquierdo-Muñoz, A., Gago, J.F., Rosselló-Mora, R., Antón, J., Ramos-Esplá, A.A., 2016. Effects of the 2015 heat wave on benthic invertebrates in the Tabarca Marine Protected Area (southeast Spain). *Mar. Environ. Res.* 122, 135–142.
- Ruel, J.J., Ayres, M.P., 1999. Jensen's inequality predicts effects of environmental variation. *Trends Ecol. Evol.* 14, 361–366.
- Russell, J.A., Moran, N.A., 2006. Costs and benefits of symbiont infection in aphids: variation among symbionts and across temperatures. *Proc. R. Soc. Lond. B. Bio.* 273, 603–610.
- Sentis, A., Hemptinne, J.L., Brodeur, J., 2013. Effects of simulated heat waves on an experimental plant–herbivore–predator food chain. *Global Change Biol.* 19, 833–842.
- Smith, M.D., 2011. An ecological perspective on extreme climatic events: a synthetic definition and framework to guide future research. *J. Ecol.* 99, 656–663.
- Sunday, J.M., Bates, A.E., Dulvy, N.K., 2012. Thermal tolerance and the global redistribution of animals. *Nat. Clim. Change* 2, 686.
- van Emden, H.F., Harrington, R., 2007. Aphids as Crop Pests. CAB International, Cambridge, Massachusetts, USA.
- Vincenzi, S., De Leo, G.A., Bellingeri, M., 2012. Consequences of extreme events on population persistence and evolution of a quantitative trait. *Ecol. Inform.* 8, 20–28.
- Walter, M.F., Petersen, N.S., Biessmann, H., 2010. Heat shock causes the collapse of the intermediate filament cytoskeleton in *Drosophila* embryos. *Genesis* 11, 270–279.
- Walther, G.R., Post, E., Convey, P., Menzei, A., Parmesan, C., Trevor, J.C., Fromentin, J.M., Hoegh-Guldberg, O., Bairlein, F., 2002. Ecological responses to recent climate change. *Nature* 416, 389.
- Welbergen, J.A., Klose, S.M., Markus, N., Eby, P., 2008. Climate change and the effects of temperature extremes on Australian flying-foxes. *P. Roy. Soc. B-Biol. Sci.* 275, 419–425.
- Wernberg, T., Smale, D.A., Tuya, F., Thomsen, M.S., Langlois, T.J., Bettignies, T., Bennett, S., Rousseaux, C.S., 2013. An extreme climatic event alters marine ecosystem structure in a global biodiversity hotspot. *Nat. Clim. Change* 3, 78.
- Zani, P., Cohnstaedt, L., Corbin, D., Bradshaw, W., Holzapfel, C., 2005. Reproductive value in a complex life cycle: heat tolerance of the pitcher plant mosquito, *Wyeomyia smithii*. *J. Evol. Biol.* 18, 101–105.
- Zhang, W., Chang, X.Q., Hoffmann, A.A., Zhang, S., Ma, C.S., 2015. Impact of hot events at different developmental stages of a moth: the closer to adult stage, the less reproductive output. *Sci. Rep.* 5, 10436.
- Zhang, W., Zhao, F., Hoffmann, A.A., Ma, C.S., 2013. A single hot event that does not affect survival but decreases reproduction in the diamondback moth, *Plutella xylostella*. *PLoS One* 8, e75923.
- Zhao, F., Hoffmann, A.A., Xing, K., Ma, C.S., 2017. Life stages of an aphid living under similar thermal conditions differ in thermal performance. *J. Insect Physiol.* 99, 1–7.
- Zhao, F., Zhang, W., Hoffmann, A.A., Ma, C.S., 2014. Night warming on hot days produces novel impacts on development, survival and reproduction in a small arthropod. *J. Anim. Ecol.* 83, 769–778.